PATENT
Attorney Docket No. **AMBER-06311**

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Kenneth J. Rothschild *et al.*
Serial No.: 09/813,197 Group No.: 1636
Filed: 3/20/01 Examiner:
Entitled: **Methods For The Detection, Analysis And Isolation Of Nascent Proteins**

PRELIMINARY AMENDMENT AND RESPONSE TO NOTICE TO FILE MISSING PARTS MAILED May 5, 2001

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. § 1.8(a)(1)(i)(A)

I hereby certify that this correspondence (along with any referred to as being attached or enclosed) is, on the date shown below, being deposited with the U.S. Postal Service with sufficient postage as first class mail in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231.

Dated: August 3, 2001 By: _____
Christopher J. Collins

Sir or Madam:

This is responsive to the Notice to File Missing Parts mailed May 5, 2001. Please amend the above referenced application as follows:

REMARKS

Applicants have amended reference to Figure 11 as set out in the specification. These amendments are designed to harmonize the description of Figure 11, in the specification, with the formatting of this same figure in the application as originally filed. Specifically, Applicants have deleted reference (in the specification) to subsections "(A), (B), and (C)" in Figure 11 given that the figure as originally filed was not labeled with these subsections.

Applicants assert these amendments to the specification do not introduce any new matter.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment in conformity with 37 C.F.R 1.121(c)(1)(i-ii). The attached page is captioned, "Version With Markings To Show Changes Made".

IN THE SPECIFICATION:

Please replace the paragraph beginning at page 19, line 22 with the following rewritten paragraph:

- - Figure 11 provides examples of non-native amino acids with reporter properties, illustrates participation of a reporter in protein synthesis, and illustrates synthesis of a reporter.- -

Please replace the paragraph beginning at page 51, line 14 with the following rewritten paragraph:

- - The chemical synthesis of a reporter can be based on the linkage of a chemical moiety or a molecular component having reporter properties with a native amino acid residue. There are many fluorescent molecules which are sensitive to their environment and undergo a change in the wavelength of emitted light and yield of fluorescence. When these chemical moieties, coupled to amino acids, are incorporated into the synthesized protein, their environments are altered because of a difference between the bulk aqueous medium and the interior of a protein which can causes reduced accessibility to water, exposure to charged ionic groups, reduced mobility, and altered dielectric constant of the surrounding medium. Two such examples are shown in Figure 11.- -

Please replace the paragraph beginning at page 52, line 14 with the following rewritten paragraph:

-- A second example of a reporter is a marker based on coumarin such as 6,7-(4', 5'-prolino)coumarin. This compound can be chemically synthesized by coupling a fluorophore like coumarin with an amino-acid structural element in such a way that the fluorophore would alter its emission or absorption properties after forming a peptide linkage (Figure 11). For example, a proline ring containing secondary amino functions

will participate in peptide bond formation similar to a normal primary amino group. Changes in fluorescence occur due to the co-planarity of the newly formed peptide group in relation to the existing fluorophore. This increases conjugation/delocalization due to the π-electrons of nitrogen-lone pair and carbonyl-group in the peptide bond. Synthesis of such compounds is based on coumarin synthesis using ethylacetoacetate (Figure 11). --

Respectfully submitted:

Dated: August 3, 2001

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Application Serial: 09/813,197

VERSION WITH MARKINGS TO SHOW CHANGES MADE
(Response to File the Notice of Missing Parts)

The paragraph beginning at line 22 on page 19 has been amended as follows:

- - Figure 11[(A)] provides examples of non-native amino acids with reporter properties, [Figure 11(B) shows] illustrates participation of a reporter in protein synthesis, and [Figure 11(C) shows] illustrates synthesis of a reporter.- -

The paragraph beginning at line 14 on page 51 has been amended as follows:

- - The chemical synthesis of a reporter can be based on the linkage of a chemical moiety or a molecular component having reporter properties with a native amino acid residue. There are many fluorescent molecules which are sensitive to their environment and undergo a change in the wavelength of emitted light and yield of fluorescence. When these chemical moieties, coupled to amino acids, are incorporated into the synthesized protein, their environments are altered because of a difference between the bulk aqueous medium and the interior of a protein which can causes reduced accessibility to water, exposure to charged ionic groups, reduced mobility, and altered dielectric constant of the surrounding medium. Two such examples are shown in Figure 11[A].- -

The paragraph beginning at line 14 on page 52, has been amended as follows:

A second example of a reporter is a marker based on coumarin such as 6,7-(4', 5'-prolino)coumarin. This compound can be chemically synthesized by coupling a fluorophore like coumarin with an amino-acid structural element in such a way that the fluorophore would alter its emission or absorption properties after forming a peptide linkage (Figure 11[B]). For example, a proline ring containing secondary amino functions will participate in peptide bond formation similar to a normal primary amino group. Changes in fluorescence occur due to the co-planarity of the newly formed peptide group in relation to the existing fluorophore. This increases conjugation/delocalization due to the π-electrons of nitrogen-lone pair and carbonyl-

group in the peptide bond. Synthesis of such compounds is based on coumarin synthesis using ethylacetoacetate (Figure 11[C]).